Electronic Supplementary Material (ESI) for RSC Sustainability.

Supporting information

Visible-light driven fumarate synthesis from pyruvate and gaseous CO_2 with the hybrid system of photocatalytic NADH regeneration and dual biocatalysts

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1. Determination for pyruvate and L-malate concentration using ion chromatography

The concentrations of pyruvate and L-malate were determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 x 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for pyruvate was detected at 8.71-9.20 min. The electrical conductivity changes in the various pyruvate concentrations (0 - 10 mM) were shown in Figure S1(a). The inset of Figure S1(a) shows the relationship between the pyruvate concentration and the detection peak area using ion chromatograph.

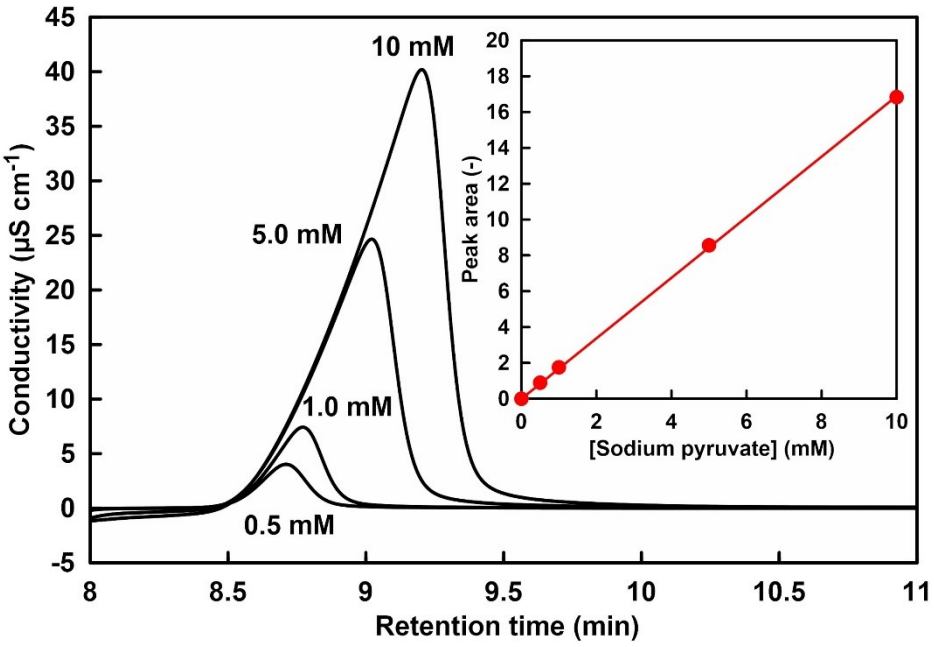

Figure S1. Chromatogram of sodium pyruvate (0 - 10 mM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the sodium pyruvate concentration and the detection peak area.

As shown in the inset of Figure S1, the sodium pyruvate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S1).

$$\text{Peak area} = 1.69 \times [\text{Pyruvate}](\text{mM}) \quad \text{(S1)}$$

The retention time for L-malate was detected at 10.11-10.13 min. The electrical conductivity changes in the various L-malate concentrations (0 – 1.0 mM) during the ion chromatograph analysis were shown in Figure S2. Inset of Figure S2 shows the relationship between the L-malate concentration and the detection peak area using ion chromatograph.

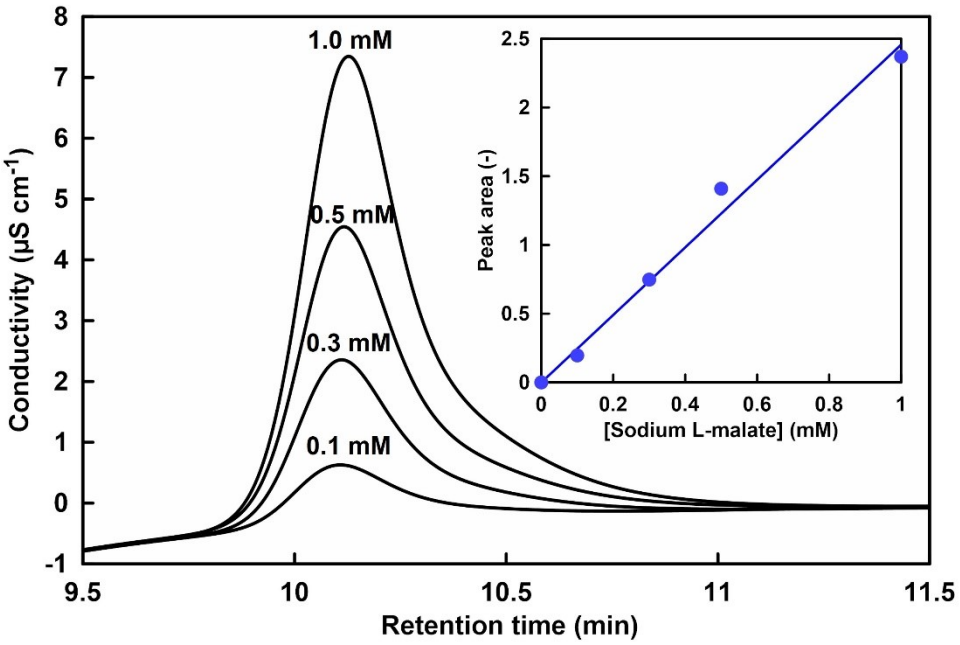

Figure S2. Chromatogram of sodium L-malate (0 - 1000 μM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the L-malate concentration and the detection peak area.

As shown in the inset of Figure S2, the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S2).

$$\text{Peak area} = 2.46 \times [\text{L-malate}](\text{mM}) \quad \text{(S2)}$$

The retention time for fumarate was detected at 12.28-12.37 min. The electrical conductivity changes in the various sodium fumarate concentrations (0 – 1.0 mM) during the ion chromatograph analysis were shown in Figure S3. Inset of Figure S3 shows the relationship between the sodium fumarate concentration and the detection peak area using ion chromatograph.

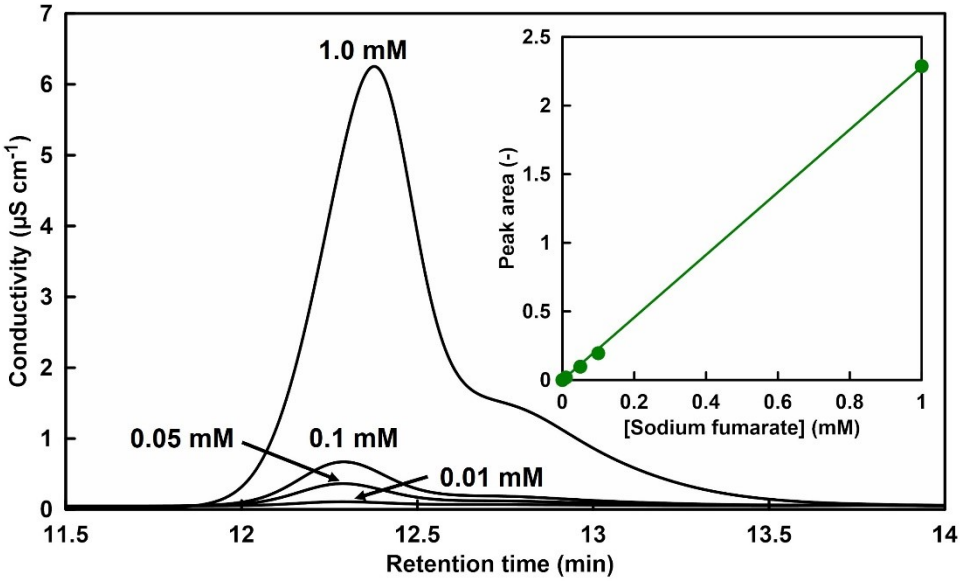

Figure S3. Chromatogram of sodium fumarate (0 – 1.0 mM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the fumarate concentration and the detection peak area.

As shown in the inset of Figure S3, the fumarate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S3).

$$\text{Peak area} = 2.28 \times [\text{fumarate}](\ \mu\text{M}) \qquad \text{(S3)}$$

2. L-Malate synthesis from the pyruvate and direct captured CO_2 with MDH

Figure S4 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and MDH (0.7 U, *ca.* 1.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.8) during the incubation.

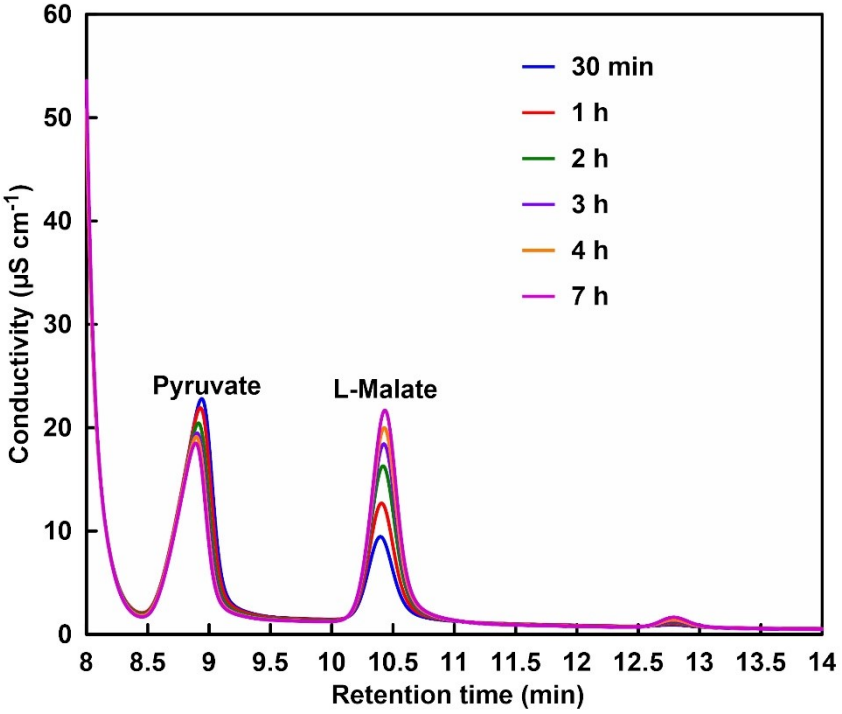

Figure S4. A chart of an ion chromatogram for pyruvate or L-malate concentration in the solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and MDH (0.7 U, *ca.* 1.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.8). The gas phase: CO_2.

3. Fumarate synthesis from the pyruvate and direct captured CO_2 with MDH and FUM

Figure S5 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM), MDH (0.7 U, *ca.* 1.6 μM) and FUM (0.5 U; 1.3 nM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.8) during the incubation.

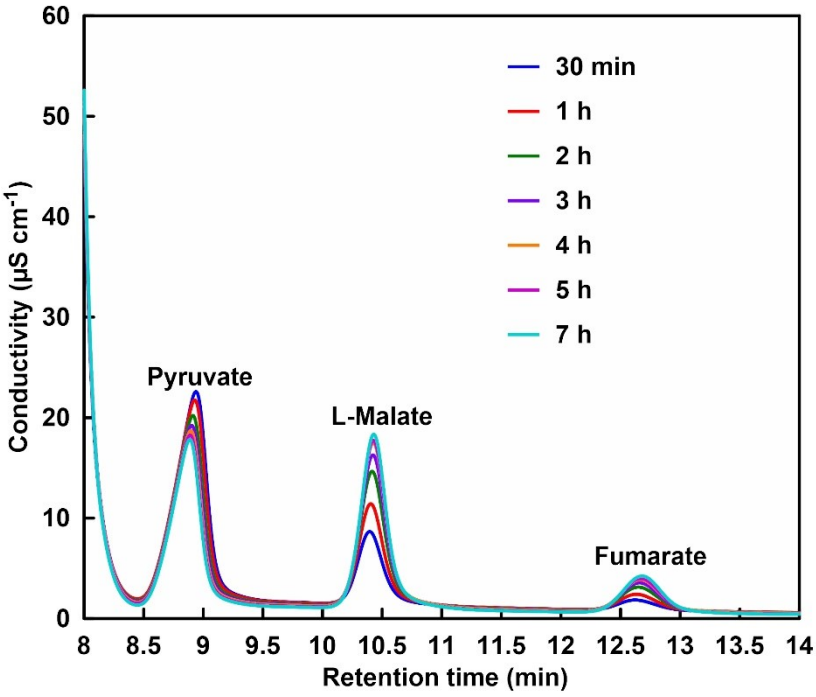

Figure S5. A chart of an ion chromatogram for pyruvate, L-malate or fumarate concentration in the solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM), MDH (0.7 U, *ca.* 1.6 μM) and FUM (0.5 U; 1.3 nM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.8). The gas phase: CO_2.

4. Visible-light driven L-malate synthesis from pyruvate and direct captured CO_2 with the system of TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and MDH

Figure S6 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+(0.5 mM) and MDH (0.7 U, *ca.* 1.6 μM) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.8) with irradiation. The gas phase was filled with CO_2 gas.

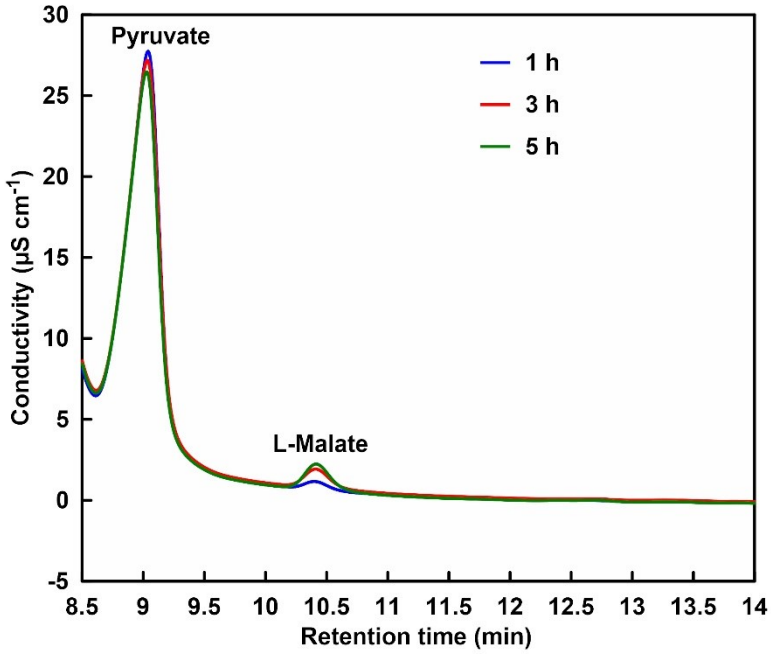

Figure S6. A chart of an ion chromatogram for pyruvate or L-malate concentration in the solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+(0.5 mM) and MDH (0.7 U, *ca.* 1.6 μM) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.8) with irradiation. The gas phase was filled with CO_2 gas.

5. Visible-light driven fumarate synthesis from pyruvate and direct captured CO_2 with the system of TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, MDH and FUM

Figure S7 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+(0.5 mM), MDH (0.7 U, *ca.* 1.6 μM) and FUM (0.5 U; 1.3 nM) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.8) under conditions with varying ratios of CO_2 and N_2 in the gas phase with irradiation.

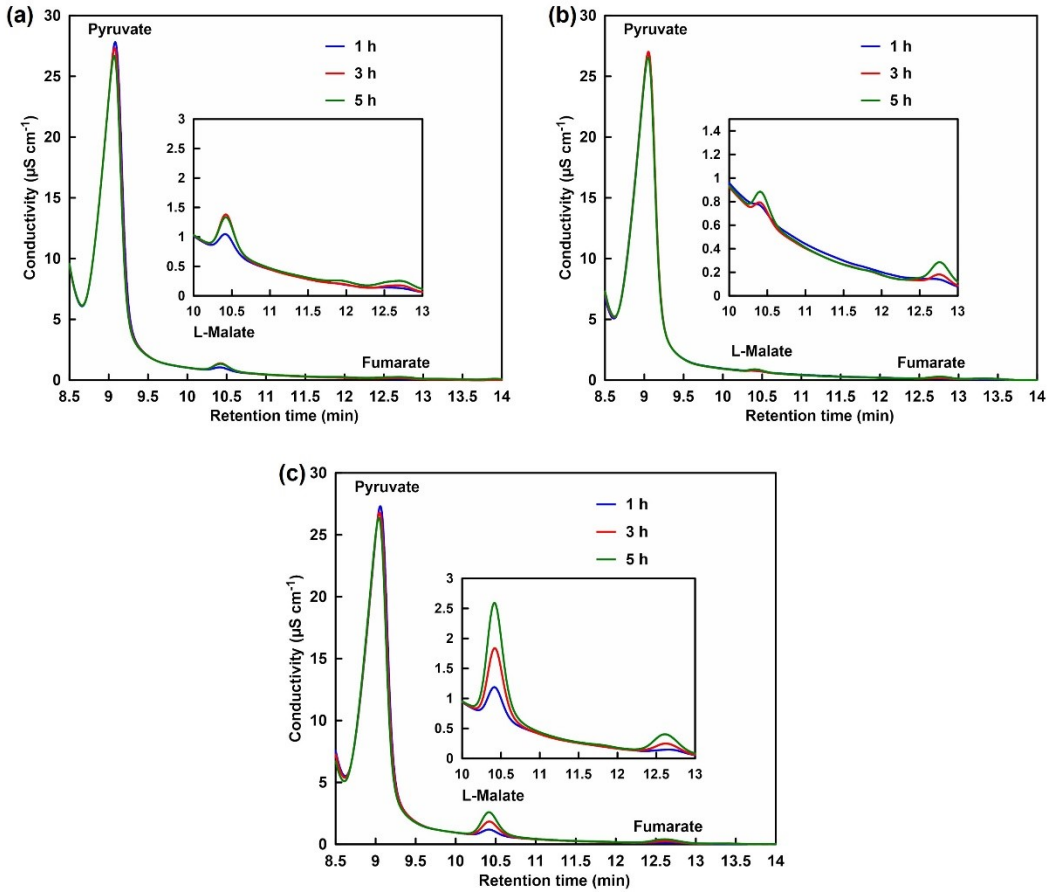

Figure S7. A chart of an ion chromatogram for pyruvate, L-malate or fumarate concentration in the solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+(0.5 mM), MDH (0.7 U, *ca.* 1.6 μM) and FUM (0.5 U; 1.3 nM) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.8) under conditions with irradiation. The gas phase : (a) 15 % CO_2 gas, (b) 50 % CO_2 gas and (c) 100 % CO_2 gas.